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VBPA

Report of the National FeLV/FIV Awareness Project

Thomas P. O'Connor, Jr. PhD; Quentin J. Tonelli, PhD; Janet M. Scarlett, DVM, PhD

The FeLV and the feline immunodeficiency virus (FIV) are the 2 most common pathogenic retroviruses reported in feline populations. Although the viruses induce disease manifestations that are clinically indistinguishable, the physical properties of the viral particles are clearly distinct.[1] The FeLV was initially described by Jarrett et al[2] in 1964 and has been classified in the Oncornaviridae subfamily of retroviruses. In addition to the oncogenic potential of the virus, FeLV infection can result in a number of degenerative and immunosuppressive disorders, including chronic gingivitis, stomatitis, oral ulcers and chronic respiratory tract disease.[3] The FIV was initially identified and isolated by Pedersen et al[4] in 1986 and has been classified in the retroviral subfamily Lentiviridae. The primary disease syndrome induced by FIV infection also is degenerative and immunosuppressive in nature and includes chronic infections of the mouth, nasal passages, and intestinal tract; lymphadenopathy; and fever.[5]

The generation of polyclonal antibodies to FeLV, and monoclonal antibodies specific for the group-associated antigen of FeLV led to the development of specific immunoassays for the detection of FeLV antigen.[2,6] The continuous propagation of FIV in an established feline cell line and the purification of FIV from cell culture led to the development of immunoassays for the detection of antibody to FIV.[4,7] Enzyme-linked immunosorbent assays, initially developed separately for the detection of FeLV antigen (Ag) and FIV antibody (Ab), were subsequently combined to construct an assay for simultaneous detection of FeLV Ag and/or FIV Ab.[8]

The purpose of the National FeLV/FIV Awareness Project was to determine the nationwide distribution of the viruses among cats with clinical signs of disease (hereafter referred to as symptomatic) and high-risk cats, to learn more about regional prevalence in symptomatic and high-risk cats, and to expand the understanding of the epidemiology of FeLV and FIV infections. Symptomatic and high-risk cats were included in the study because these groups would more likely be examined by veterinary practitioners for possible infection. We herein report a serologic survey of 27,976 cats from all 50 states of the United States. Individual veterinary practitioners tested cats that they considered to be symptomatic or at high risk for infection, using the combination FeLV Ag/FIV Ab test. In addition to assay results, pertinent data were requested on a survey form that included health status, gender, age, breed, environment, number of household cats, and categorization of clinical signs of disease.

Materials and Methods

FeLV Ag/FIV Ab ELISA method—The combination FeLV Ag/FIV Ab ELISA can be performed using serum, plasma or blood. The assay procedure was included in the package insert supplied with each test kit.

The sensitivity and specificity of the combination FeLV Ag/FIV Ab ELISA were initially evaluated by testing samples from a population of experimentally infected cats and samples obtained from cats in a field setting (n = 324). Samples were analyzed using a licensed FeLV Ag microtitration plate test[b] and a licensed FIV Ab microtitration plate test.[c] Samples positive for FeLV Ag were confirmed by use of antibody neutralization assays, and FIV Ab-positive samples were confirmed positive by use of western blot and sodium dodecyl sulfate radioimmunoprecipitation assays. Test sensitivity was 100% for FeLV Ag and 98.3% for FIV Ab. The FIV Ab confirmatory assays were validated using samples with known positive or negative results.[7] The overall test specificity was 100% for FeLV Ag and for FIV Ab.

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[a]CITE Combo FeLV Ag/FIV Ab, IDEXX Laboratories Inc, Westbrook, Me.

[b]PetChek FeLV Ag test kit, IDEXX Laboratories Inc, Westbrook, Me.

[c]PetChek FIV Ab test kit, IDEXX Laboratories Inc, Westbrook, Me.

Table 1—Prevalence of FeLV and feline immunodeficiency virus (FIV) seropositivity by state

State	Total	No. FeLV Ag-positive (%)	No. FIV Ab-positive (%)	No. FeLV Ag-and FIV Ab-positive (%)
AK	72	7 (9.7)	3 (4.2)	0 (0.0)
AL	415	60 (14.5)	26 (6.3)	5 (1.2)
AR	58	7 (12.1)	6 (10.3)	0 (0.0)
AZ	534	86 (16.1)	39 (7.3)	14 (2.6)
CA	3,427	353 (10.3)	190 (5.5)	23 (0.7)
CO	358	54 (15.1)	33 (9.2)	10 (2.8)
CT	375	32 (8.5)	22 (5.9)	5 (1.3)
DE	20	3 (15.0)	2 (10.0)	1 (5.0)
FL	2,643	339 (12.8)	223 (8.4)	53 (2.0)
GA	618	89 (14.4)	43 (7.0)	5 (0.8)
HI	158	16 (10.1)	25 (15.8)	1 (0.6)
IA	161	29 (18.0)	8 (5.0)	2 (1.2)
ID	61	11 (18.0)	5 (8.2)	1 (1.6)
IL	931	95 (10.2)	72 (7.7)	14 (1.5)
IN	627	93 (14.8)	57 (9.1)	19 (3.0)
KS	345	43 (12.5)	29 (8.4)	5 (1.4)
KY	291	38 (13.1)	18 (6.2)	3 (1.0)
LA	205	39 (19.0)	28 (13.7)	6 (2.9)
MA	957	124 (13.0)	82 (8.6)	11 (1.1)
MD	213	22 (10.3)	13 (6.1)	2 (0.9)
ME	529	71 (13.4)	43 (8.1)	8 (1.5)
MI	1,095	125 (11.4)	65 (5.9)	14 (1.3)
MN	262	25 (9.5)	7 (2.7)	0 (0.0)
MO	288	43 (14.9)	21 (7.3)	2 (0.7)
MS	144	20 (13.9)	13 (9.0)	0 (0.0)
MT	59	3 (5.1)	2 (3.4)	1 (1.7)
NC	843	165 (19.6)	71 (8.4)	18 (2.1)
ND	24	2 (8.3)	1 (4.2)	0 (0.0)
NE	113	22 (19.5)	10 (8.8)	2 (1.8)
NH	271	39 (14.4)	31 (11.4)	6 (2.2)
NJ	473	71 (15.0)	35 (7.4)	10 (2.1)
NM	168	17 (10.1)	8 (4.8)	0 (0.0)
NV	89	9 (10.1)	2 (2.2)	0 (0.0)
NY	1,652	227 (13.7)	119 (7.2)	21 (1.3)
OH	1,456	161 (11.1)	86 (5.9)	19 (1.3)
OK	135	30 (22.2)	18 (13.3)	3 (2.2)
OR	945	143 (15.1)	72 (7.6)	15 (1.6)
PA	617	146 (23.7)	60 (9.7)	12 (1.9)
RI	50	4 (8.0)	9 (18.0)	0 (0.0)
SC	134	23 (17.2)	15 (11.2)	3 (2.2)
SD	25	5 (20.0)	6 (24.0)	2 (8.0)
TN	296	49 (16.6)	30 (10.1)	5 (1.7)
TX	2,330	318 (13.6)	211 (9.1)	51 (2.2)
UT	134	14 (10.4)	9 (6.7)	0 (0.0)
VA	805	103 (12.8)	33 (4.1)	8 (1.0)
VT	146	21 (14.4)	7 (4.8)	3 (2.1)
WA	1,194	178 (14.9)	101 (8.5)	28 (2.3)
WI	583	62 (10.6)	28 (4.8)	3 (0.5)
WV	62	17 (27.4)	8 (12.9)	2 (3.2)
WY	96	15 (15.6)	8 (8.3)	3 (3.1)
*	489	44 (9.0)	26 (5.3)	3 (0.6)
Total	27,976	3,712 (13.3)	2,079 (7.4)	422 (1.5)

Cats seropositive for both viruses were included in the FeLV Ag and FIV Ab summations.

Data for the state of Virginia include Washington DC. *State information not provided.

Design of project—Veterinary practitioners and clinics were informed of the project by a direct mail campaign to 12,500 veterinarians, and by veterinary distributor representatives throughout the United States. Participating practitioners purchased test kits, were given information about the project, and were asked to complete an epidemiologic survey form. Each practitioner was asked to identify, test, and report results for only symptomatic and high-risk cats. Signs suggestive of FeLV or FIV infection were not specified. High-risk cats were described as outdoor cats, cats exposed to outdoor cats, and cats in large multicat households with frequent introduction of new cats.

Table 2—Regional comparison of FeLV and FIV seropositivity

Region	Total	No. FeLV Ag-positive (%)	No. FIV Ab-positive (%)	No. FeLV Ag/FIV Ab-positive (%)
Northwest (WA, OR, MT, ID, WY, AK)	2,427	357 (14.7)	191 (7.9)	48 (2.0)
Southwest (CA, NV, UT, AZ, CO, HI)	4,700	532 (11.3)	298 (6.3)	48 (1.0)
South Central (NM, TX, OK, AR, LA)	2,896	411 (14.2)	271 (9.4)	60 (2.1)
Midwest (ND, SD, NE, KS, MN, IA, MO)	1,218	169 (13.9)	82 (6.7)	13 (1.0)
Lakes (WI, IL, IN, MI, OH)	4,692	536 (11.4)	308 (6.6)	69 (1.5)
East (PA, MD, NJ, DE, NY)	2,975	469 (15.8)	229 (7.7)	46 (1.5)
Northeast (ME, NH, VT, MA, RI, CT)	2,328	291 (12.5)	194 (8.3)	33 (1.4)
Midatlantic (WV, VA, KY, NC, TN)	2,297	372 (16.1)	160 (7.0)	36 (1.6)
South (MS, AL, GA, SC, FL)	3,954	531 (13.4)	320 (8.1)	66 (1.7)
*Other	489	44 (9.0)	26 (5.3)	3 (0.6)
Total	27,976	3,712 (13.3)	2,079 (7.4)	422 (1.5)

*State information not provided.

Information requested for each cat included test results, gender, breed, age, number of cats in household, environment of the cat (indoor, outdoor, or both), health status (symptomatic or asymptomatic [not manifesting clinical signs of disease]) and presence of specified clinical signs of disease. The specified clinical signs of disease were fever, weight loss, abscesses, diarrhea, swollen lymph nodes, dermatitis, oral infections, rhinitis, conjunctivitis, dehydration, urinary tract infection and neurologic abnormalities. A total of 1,558 veterinarians responded to the survey. Completed survey forms were returned, and results were compiled.

Statistical methods—Comparisons of the prevalence of infection of FeLV and/or FIV among subgroups of the population were made using a χ^2 test of independence. Probability value <0.01 was considered statistically significant.

Results

National distribution of FeLV Ag- and FIV Ab-positive results— The survey involved 27,976 domestic cats from across the United States that were considered by veterinary practitioners to be symptomatic or to be at risk for FeLV or FIV infection. In the overall study population, 13.3% were positive for FeLV Ag, 7.4% were positive for FIV Ab, and 1.5% were positive for both viruses. The prevalence of FeLV Ag and FIV Ab in the symptomatic/at-risk population was calculated in a state-by-state fashion, and was found to be fairly uniform throughout the nation (Table 1). The states were subse-

Table 3—Basic features of population

Category	Total No.	No. FeLV Ag-positive (%)	No. FIV Ab-positive (%)	No. FeLV Ag- and FIV Ab-positive (%)*
Health status†				
Asymptomatic, at risk	15,374	1,049 (6.8)	619 (4.0)	82 (0.5)
Symptomatic	12,602	2,663 (21.1)	1,460 (11.6)	340 (2.6)
Gender				
Female	12,517	1,391 (11.1)	445 (3.6)	86 (0.7)
Male	15,459	2,321 (15.0)	1,634 (10.6)	336 (2.2)
Age (yr)				
<1	9,715	620 (6.4)	144 (1.5)	19 (0.20)
1 to 3	8,656	1,581 (18.3)	609 (7.0)	157 (1.8)
4 to 6	3,331	608 (18.3)	420 (12.6)	99 (3.0)
>6	4,171	559 (13.4)	686 (16.4)	103 (2.5)
Age not reported	2,103	344 (16.2)	220 (10.5)	44 (2.1)
Environment				
Indoor	5,588	419 (7.5)	150 (2.7)	26 (0.5)
Outdoor	2,640	539 (20.4)	341 (12.9)	74 (2.8)
Both	12,470	1,933 (15.5)	1,125 (9.0)	231 (1.9)
Unknown	7,278	821 (11.3)	463 (6.4)	91 (1.2)
Breed				
Domestic	18,736	2,468 (13.2)	1,368 (7.4)	283 (1.5)
Mixed breed/ unknown	7,143	1,032 (14.4)	595 (8.3)	115 (1.6)
Purebreed	2,097	212 (10.1)	96 (4.6)	24 (1.1)
Total population	27,976	3,712 (13.3)	2,079 (7.4)	422 (1.5)

*Cats seropositive for both viruses were included in the FeLV Ag and FIV Ab summations. †Asymptomatic = cats not manifesting signs of disease; symptomatic = cats manifesting signs of disease.

Table 4—Comparison of FeLV Ag-, FIV Ab-, and FeLV Ag-FIV Ab-positive cats

Category	Overall population	FeLV Ag-positive	FIV Ab-positive	FeLV Ag/FIV Ab-positive
Health status				
Ratio of symptomatic, at-risk to asymptomatic	0.82:1	2.5:1	2.3:1	4.1:1
Gender				
Ratio of male to female	1.2:1	1.7:1	3.7:1	3.9:1
Environment				
Ratio of outdoor to indoor	0.5:1	1.2:1	2.1:1	2.8:1
Mean age (yr)	2.5	2.9	5.2	4.25

See Table 3 for key.

quently grouped into regions, and seroprevalence was determined for various regions of the country (Table 2). Although the regional seropositive rate differences were small, some were found to be significant ($P < 0.01$).

Epidemiologic features of the population—A total of 12,602 cats (45.0%) was categorized as symptomatic and of these, 2,663 (21.1%) were FeLV Ag-positive and 1,460 (11.6%) were FIV Ab positive (Tables 3 and 4). The seropositive rates for cats categorized as asymptomatic/at-risk were 6.8% (FeLV Ag) and 4.0% (FIV Ab). The ratio of cats categorized as symptomatic to cats categorized as asymptomatic/at-risk was slightly higher for FeLV Ag-positive cats (2.4:1), compared with FIV Ab-positive cats (2.1:1).

In the overall symptomatic/at-risk population of cats were 12,517 females and 15,459 males (male to female ratio, 1.2:1). A total of 11.1% of the females and 15.0% of the males were FeLV Ag-positive, whereas 3.6% of the females and 10.6% of the males were FIV Ab-positive. Male-to-female ratio for seropositive cats was 1.7:1 (FeLV Ag) and 3.7:1 (FIV Ab). Ages of the FeLV Ag- and the FIV Ab-positive cats ranged from 1 month to >15 years. The prevalence of FeLV infection was essentially the same for cats in the age groups 1 to 3 years and 4 to 6 years, and was significantly ($P < 0.01$) reduced in the groups of cats <1 year old or >6 years old. In contrast, the prevalence of FIV and FIV-FeLV infection was significantly ($P < 0.01$, χ^2 test of trend) increased with age. Mean age of the overall study population was 2.5 years, mean age of the seropositive populations was 2.9 (FeLV Ag) and 5.2 (FIV Ab) years. Mean age of the seropositive symptomatic cats was greater than mean age of the seropositive asymptomatic cats. This difference was 1.3 years for FeLV Ag-positive cats and 1.8 years for FIV Ab-positive cats.

Seroprevalence for FeLV Ag and FIV Ab was significantly ($P < 0.01$) lower for indoor cats, compared with outdoor cats. Cats that were classified as both (indoor and outdoor) or as unknown had seroprevalence that was between that of the strictly outdoor and strictly indoor categories. Domestic and mixed-breed cats had essentially the same seroprevalence, which was significantly ($P < 0.01$) higher than the comparable value for purebred cats.

Seroprevalence was determined for each virus in groups of cats selected according to the number of household cats living in the same domain as the cat initially submitted for testing. The FeLV Ag seropositive rate increased as the number of cats in the household increased (Table 5). The FeLV Ag and FIV Ab-seropositive rates were significantly ($P < 0.01$) higher in cats from households with more than 6 cats, compared with households with either 1 or 2 to 5 cats.

A total of 422 cats (1.5% of the overall study population) was found to be infected with both viruses. These cats were more likely to be clinically ill, more likely to be male, and more likely to be outdoor cats than were cats infected by either virus alone. In addition, cats positive for FeLV Ag were more likely to be FIV Ab-positive than were FeLV Ag-negative cats. Among the 3,712 cats that were positive for FeLV Ag, 422 (11.4%) were positive for FIV Ab. This rate was significantly ($P < 0.01$) higher than the FIV Ab seroprevalence of 6.8% found for the FeLV Ag-negative population.

Table 5—Infection rate in multicat households

No. of household cats	Total No.	No. FeLV Ag-positive (%)	No. FIV Ag-positive (%)	No. FeLV Ag/FIV Ab-positive (%)
1	12,194	1489 (12.2)	910 (7.5)	170 (1.4)
2 to 5	12,240	1648 (13.5)	852 (7.0)	181 (1.5)
>6	2,272	366 (16.1)	225 (9.9)	48 (2.1)
Not specified	1,270	209 (16.4)	92 (7.2)	23 (1.8)

Table 6—Sexually intact vs neutered cats

Gender	Total No.	No. FeLV Ag-positive (%)	No. FIV Ab-positive (%)
Female (sexually intact)	7,274	726 (10.0)	136 (2.7)
Female (neutered)	5,243	665 (12.7)	249 (4.7)
Male (sexually intact)	8,423	1,203 (14.3)	684 (8.1)
Male (neutered)	7,036	1,118 (15.9)	950 (13.5)

Table 7—Rate of FIV Ab positivity in low-risk populations

Low-risk groups	No.	No. FIV Ab-positive (%)
Asymptomatic, indoor only, single cat household	1,450	21 (1.4)
Group 1/sexually intact	1,057	9 (0.85)
Group 1/<2 years old	955	7 (0.73)

(n = 24,264). Cats that were FeLV Ag-positive were 1.7 times more likely to be FIV Ab-positive than were FeLV Ag-negative cats.

Prevalence of infection for sexually intact and neutered cats was determined (Table 6). Seropositive rates for FeLV Ag and FIV Ab were greater for neutered females and neutered males compared with their sexually intact counterparts. Mean age of the neutered cats (female and male combined) was compared with mean age of the sexually intact cats (female and male combined) for FeLV Ag- and FIV Ab-positive cats. Mean age of the FeLV Ag-positive neutered and sexually intact cats was 4.2 and 1.9 years, respectively. Mean age of the FIV Ab-positive neutered and sexually intact cats was 6.5 and 3.3 years, respectively. Prevalence of FIV Ab was found for several groups of cats predicted to be at lower risk for infection. A total of 21 FIV Ab-positive results (1.4%) were reported for the 1,450 cats in the low-risk group comprising asymptomatic, indoor only, single-cat household animals (Table 7). This population was further divided to give 2 additional groups predicted to be at lower risk. The first consisted of 1,057 cats and contained only sexually intact cats (female and male) from the aforementioned population. The second subgroup was made up of 955 cats <2 years old, from the same population. Nine (.85%) of the sexually intact cats and 7 (0.73%) of the cats <2 years old were positive for FIV Ab.

The frequency of specified clinical signs of disease was determined in descending order of frequency of FeLV-positive cats (Table 8). Among the 12,602 symptomatic cats, 8,317 were reported with the clinical history section of the survey form completed. Of this group, 1,574 (18.9%) were infected with FeLV alone, 777 (9.3%) were infected with FIV alone, and 249 (3.0%) were infected with both retroviruses. Among the FeLV-infected cats, weight loss (63.3%), fever (41.9%) and dehydration (35.2%) were observed most frequently. Weight loss (51.0%), fever (36.2%) and oral cavity infections (26.7%) were the most commonly reported signs of disease for FIV-infected cats. Oral

*Table 8—Clinical signs of disease in FeLV Ag-, FIV Ab- and FeLV Ag/FIV Ab-positive cats**

Description	No. FeLV Ag-positive (%)	No. FIV Ab-positive (%)	No. FeLV Ag/FIV Ab-Positive (%)
No. of symptomatic cats with disease history	1,574	777	249
Weight loss	996 (63.3)	394 (51.0)	173 (69.4)
Fever	660 (41.9)	281 (36.2)	107 (43.0)
Dehydration	555 (35.2)	162 (21.0)	115 (46.1)
Rhinitis	279 (17.7)	151 (19.4)	56 (22.5)
Diarrhea	271 (17.2)	87 (11.2)	35 (14.1)
Conjunctivitis	271 (17.2)	148 (19.0)	47 (18.9)
Oral infections	238 (15.1)	208 (26.8)	63 (25.3)
Swollen lymph nodes	202 (12.8)	79 (10.2)	31 (12.4)
Abscesses	186 (11.8)	126 (16.2)	33 (13.3)
Signs of neurologic dysfunction	80 (5.1)	36 (4.6)	18 (7.2)
Dermatitis	67 (4.3)	95 (12.2)	20 (8.0)
Urinary tract infection	63 (4.0)	38 (4.9)	6 (2.4)

*Total No. of symptomatic cats with adequate clinical history was 8,317
See Table 3 for key

cavity infections were observed in substantially greater number of the FIV- (26.7%) and co-infected (25.3%) cats than in FeLV-infected (15.1%) cats. Rhinitis, conjunctivitis, swollen lymph nodes, and abscesses were each reported in approximately the same percentage for FIV-, FeLV-, and FIV/FeLV-infected cats.

Discussion

Prevalence of FeLV Ag and FIV Ab positives was measured in high-risk asymptomatic and symptomatic feline populations throughout the United States to find and compare the rates of infection in all major regions of the country. Both viruses were found to be prevalent in feline populations throughout the country. Although small state and regional differences in seroprevalence were found, it is difficult to ascertain whether these reflect actual differences in the extent of viral infection or are a consequence of unrelated factors.

Prevalence of FIV infection in the 15,374 high-risk asymptomatic and 12,602 symptomatic cats was 4.0 and 11.6%, respectively. In a recent similar serosurvey, using low or unknown risk asymptomatic (n = 511) and symptomatic cats (n = 2,254) from the continental United States and Canada, infection rates were 1.2 and 14%, respectively.[8] Seroprevalence for the 2 symptomatic cat populations was similar. The higher seroprevalence in the asymptomatic population reported in our study most likely reflects differences in the 2 asymptomatic feline populations. In the previously reported survey, asymptomatic cats without additional risk factors or with unknown risk factors were tested, whereas in our study, the asymptomatic population contained cats principally with specified risk factors for FIV infection. The seroprevalence of FIV Ab, recently reported from several countries for asymptomatic and symptomatic cats respectively, was 6 and 19% (United Kingdom), and 12.4 and 43.9% (Japan).[9,10] The significantly higher rates found for Japanese cats is

thought to reflect the high density of cats in Japan and their free-roaming lifestyle.[10]

Results of this serosurvey generally confirm many of the epidemiologic observations made in previous surveys of FIV Ab.[8-10] These include the increased infection rate found in male cats, compared with female cats, and the increased rate of infection for outdoor cats, compared with indoor cats. Both observations reflect the increased tendency of outdoor, male cats to be involved in behaviors that include biting. The FIV has been isolated from the saliva of infected cats, and saliva-mediated transmission of the virus through biting has been implicated as a major mode of infection.[8] The increased prevalence of FIV infection in older cats has been reported.[7,9] This does not appear to reflect age-related susceptibility to infection, but is a consequence of the extended latent period after exposure to the virus which has been observed in cats experimentally infected with FIV.[11] The period of apparent viral latency is typical of lentiviral infections and frequently precedes development of clinical abnormalities.[12] The increased prevalence of FIV infection in households with more than 6 cats reflects the greater contact of these cats with a large number of cats. Similar results have been reported.[9]

Prevalence of FeLV infection found in the high-risk asymptomatic and symptomatic cats was 6.8 and 21.1%, respectively. Similar seropositive rates have been reported in surveys conducted using the ELISA method.[13] A recent serosurvey conducted in the United States found seropositive rates of 3.6 and 11.3% for healthy (n = 361) and sick cats (n = 226), respectively.[13] The lower prevalence of infection in that study most likely reflects differences in survey populations. In that study, healthy cats had no known risk factors for FeLV infection and sick cats were not selected on the basis of signs suggesting disease. In our study, the population consisted principally of asymptomatic cats, selected on the basis of specified risk factors for FeLV and FIV infection, and of symptomatic cats selected on the basis of signs of disease.

In this survey, the ratio of symptomatic to asymptomatic FeLV-positive cats, the age distribution of infected cats, and the ratio of infected males to infected females were similar to reported results.[9,13] A greater percentage of outdoor cats (20.4%) than indoor cats (7.3%) was found to be infected with FeLV. The rate of FeLV infection also increased as the number of cats in the household of the test cat increased, and likely was a result of greater degree of contact of these cats with a number of different cats. Decreased prevalence of FeLV and FIV infection in purebred cats, compared with mixed-breed or domestic cats, has been reported in several studies.[8,9] This is not thought to be attributable to genetic factors, but rather is a reflection of the lifestyles of such cats. Purebred cats are more likely to be housed indoors and are less likely to have contact with seropositive cats.

Prevalence of FIV infection in the 3,712 FeLV Ag-positive cats in the overall survey was 11.4%. Frequency of FIV infection in the FeLV Ag-negative population was 6.8%. Increased prevalence of dual infection in these cats may reflect increased tendency of this group of cats to engage in behavior that would transmit FeLV and FIV, perhaps through biting. The increased seropositive rate might also reflect the diminished capacity of the immune system. Cats previously infected by one retrovirus may be less able to recover after exposure to a second virus.

Increased prevalence of positive FeLV Ag and FIV Ab results in neutered cats, compared with sexually intact cats, reflects the greater age of the neutered cats. Mean age of the FeLV Ag-positive neutered cats was 2.3 years greater than that of the FeLV Ag-positive sexually intact cats. Similarly, mean age of the FIV Ab-positive neutered cats was 3.2 years greater than that of the FIV Ab-positive sexually intact cats. Increased prevalence in the neutered cats would be predicted on the basis of age difference alone, using data given in Table 3.

Prevalence of FIV Ab for the minimal risk populations was low. In addition to providing insight on rate of infection, seropositive rates for these low-risk populations is a reliable measurement of the maximal false-positive rate of the FIV Ab test. The percent positive rate for the minimal risk subgroups (Table 7) was 0.85% for the sexually intact subgroup and 0.73% for the subgroup <2 years old. The percent positive rate for known negative groups would be equal to or less than these values. These seropositive rates indicate that the FIV Ab test specificity is >99%. These data provide a practical estimate of the test specificity because they were calculated from results collected and reported by a wide variety of veterinary practitioners in the field using instructions supplied with the test kit.

Although the sampling for this survey was not truly random, results of this serosurvey document the widespread prevalence of FeLV and FIV infections in the high-risk national feline population. Overall, 5,791 cats (20.6%) of a total of 27,976 at-risk and symptomatic cats were infected with one or both retroviruses. The substantially higher prevalence of viral infection in the symptomatic cat population apparently reflects the disease-causing potential of the viruses. In this symptomatic population of cats (n = 12,602), 32.7% or almost 1 in 3 were found to be infected by FeLV, FIV, or both viruses.

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Hematologic abnormalities in cats seropositive for feline immunodeficiency virus

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Feline immunodeficiency virus (FIV), a retrovirus (subfamily, Lentivirinae), is associated with an immunodeficiency syndrome in domestic cats.[1] After a prolonged period when clinical signs of disease are not manifested (eg, months to years), FIV infection is characterized by a variety of chronic disorders, opportunistic infections, and neoplasia.[2,3] Morphologic and biologic properties of FIV closely resemble those of human immunodeficiency virus (HIV), a lentivirus that is the etiologic agent of acquired immunodeficiency syndrome (AIDS) in human beings.[1,2,4] Hematologic abnormalities are a common feature of HIV infection.[5–11] Although lymphopenia attributable to depletion of CD4-positive (helper inducer) cells is the most prominent abnormality, other cytopenias are often seen. The prevalence of hematologic disorders increases with progression of HIV-associated disease.[8] In addition, drug-induced neutropenia is frequent in AIDS patients treated with antimicrobial agents, including trimethoprim-sulfamethoxazole, pyrimethamine-sulfadoxine, and pentamidine.[12–14] This adverse reaction contrasts to that of drugs that induce bone marrow suppression in HIV-seropositive and noninfected individuals. The pathogenesis of these HIV-associated hematologic abnormalities has yet to be elucidated.

The hematologic status of cats with naturally acquired FIV infection has been recently studied.[9,13,15] Cats with clinical signs of disease have blood and bone marrow abnormalities comparable to those reported in HIV-seropositive human beings.[13] Furthermore, FIV-infected cats appear to have increased incidence of drug (griseofulvin)-induced neutropenia.[16]

Blood Abnormalities

Hematologic variables, including CBC, total and differential WBC counts, and platelet numbers, were assessed in 53 cats referred to the clinic because of spontaneous FIV infection.[13] Diagnosis of FIV infection was based on detection of FIV-specific antibodies in serum, using ELISA[a] and immunoblot analysis (Western blot).[3] Eighteen cats (34%) were coinfected with FeLV as determined by either ELISA[b] or immunofluorescent antibody testing.[c]

Hematologic abnormalities were detected in 40 of 53 (75%) cats. Cytopenias were the most prevalent findings (Table 1). Overall, anemia, lymphopenia, neutropenia, and thrombocytopenia were observed in 36, 53, 34, and 8% of cats,

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[a] FTLV PetChek ELISA, IDEXX Corp, Portland, Me.

[b] FeLV PetChek Screening/Confirmatory ELISA, IDEXX Corp, Portland, Me.

[c] FeLeuk test, National Veterinary Laboratory, Franklin Lakes, NJ.

Ten-year study comparing enzyme-linked immunosorbent assay with the immunofluorescent antibody test for detection of feline leukemia virus infection in cats

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Summary: *Immunodetection tests for feline retroviruses are powerful tools used in modern veterinary practice. Veterinarians must fully understand the characteristics—strengths and weaknesses—of the FeLV tests so that the information gained from them can be used properly. Any FeLV ELISA or immunofluorescent antibody (IFA) test is a method for detection of FeLV infection (the virus) and is not a diagnostic test for leukemia or other feline disease.*

From previous studies, it was determined that the most accurate test for detection of persistent FeLV infection is the IFA test, which detects FeLV antigens in cytoplasm of leukocytes in the blood of infected cats. In the study reported here, 1,142,600 FeLV IFA tests were performed between June 1972 and December 1990. During this period 19.8% of the IFA test results were positive and 78% were negative. Evaluation was not possible for the remaining 2.2% of the tests because of lack of enough leukocytes in the smears to evaluate, or nonspecific staining reactions.

In 1979, 7 years after introduction of the IFA test, in-hospital FeLV ELISA were introduced, which enabled veterinarians to test for FeLV in their hospitals. Ever since that time, continual discrepancies have been reported between results of FeLV ELISA and IFA tests, particularly between positive ELISA results and their IFA test confirmation. A 10-year comparison was made between practitioner-performed in-hospital FeLV ELISA (n = 20, 240 tests) results and FeLV IFA test performed by a commercial laboratory. All samples tested by ELISA were submitted (for confirmation of results) by veterinarians from the United States, Canada, Europe, Japan, and Australia. There was 86.9% agreement between negative ELISA results and the IFA test result, but only 46.3% agreement between positive ELISA results and the IFA test result. Overall agreement was 49.0% for all confirmatory IFA tests. However, there was bias toward confirmation of positive ELISA results because 93% of confirmatory tests requested were of ELISA-positive samples.

It is apparent from this study that between 26 and 69% of in-hospital positive ELISA results and 13% of in-hospital negative ELISA results are incorrect. On the basis of these observations, we recommend that veterinarians immediately confirm all FeLV positive ELISA results by IFA testing. In addition, negative ELISA results in cats that the veterinarian suspects have FeLV infection or FeLV-induced disease should also be confirmed by IFA testing. We also recommend that all cats be vaccinated for FeLV and that they be tested for FeLV and the feline immunodeficiency virus at time of first vaccine dose.

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Retroviruses are 1 of the most important family of viruses that infect animals. Retroviral infections are often life-long chronic infections; healthy animals can carry these viruses for long periods and spread the viruses to other animals before they develop disease. Immunodetection tests for animal retroviruses are powerful tools in modern veterinary medicine, and such tests are available for the following retroviruses: FeLV, feline immunodeficiency virus (FIV), avian leukosis virus, bovine leukemia virus, and equine infectious anemia virus.

In 1972, we introduced the first routinely available test for any retrovirus, the immunofluorescent antibody (IFA) test for detection of FeLV infection in pet cats.[1] This test has been used to elucidate all FeLV-induced diseases, the biology of FeLV, and the pathogenesis of FeLV infection.[1-19] Since then, several FeLV ELISA, in various formats, have been introduced to veterinary medicine.[20-29] Currently, some of the ELISA can be performed in <20 minutes and contain disposable receptacles. Several of the ELISA kits were compared with the IFA test by the manufacturers and others, with claims of good agreement between the tests.[20-23,26-28]

Almost as soon as the first FeLV ELISA kit was introduced in 1979, discordant FeLV ELISA and IFA test results were obtained, especially between positive-ELISA results and confirmatory IFA test results.[18,20-23,26-27] Considerable controversy and confusion have arisen relating to discordant ELISA-

Table 1—Summary of FeLV immunofluorescent antibody (IFA) test results over 19 years*

Year	Results (%)		
	Positive	Negative	No evaluation
1972	32.9	66.5	0.6
1973	32.9	66.5	0.6
1974	32.8	64.8	2.4
1975	32.9	64.3	2.8
1976	29.6	67.6	2.8
1977	28.5	69.2	2.3
1978	26.4	71.3	2.3
1979	23.8	72.6	3.6
1980	23.5	73.4	2.8
1981	21.8	70.6	3.1
1982	21.5	76.0	2.5
1983	20.5	77.4	2.1
1984	19.3	79.2	1.5
1985	9.7	89.0	1.3
1986	11.4	87.0	1.6
1987	11.9	86.4	1.7
1988	13.1	84.6	2.3
1989	14.8	83.4	1.8
1990	14.9	83.2	1.9
Total tests 1,142,600	19.8	78.0	2.2

*Performed by laboratory A.

Table 2—Summary of a ten-year comparison of FeLV ELISA and IFA tests, Sept 1979 through Aug 1989

In-hospital ELISA* results	No. tested	IFA Test results†		Percentage agreement (%)
		Negative	Positive	
Negative	1,332	1,158	174	86.9
Positive	18,908	10,147	8,761	46.3
Total	20,240	Overall agreement		49.0

*ELISA were performed by veterinary practitioners (n = 18,828 tests) or by veterinary diagnostic laboratories (n = 1,412 tests). Submissions are biased toward ELISA-positive result confirmations. †Performed by laboratory A on blood smears submitted by veterinary practitioners or local veterinary laboratories.

positive, IFA-negative test results.[24,43] Because of the continual problem of discrepant test results, we conducted a 10-year comparative study of 20,240 samples, tested by ELISA, results of which were confirmed or compared with results of our FeLV IFA test.[a] The purpose of the study reported here was to ascertain the degree of concordance between in-hospital performed FeLV ELISA and the IFA test to formulate algorithms and recommendations for use of FeLV tests and management of ELISA-positive cats.[44,45] The IFA test is considered the reference standard (ie, the test against which the accuracy of other tests for infection with a specific agent is judged) for FeLV infection.

Materials and Methods

Immunofluorescent antibody test procedure—Most of the IFA FeLV testing services were performed at laboratory A[a] between June 1, 1972 and December 31, 1990 (Table 1) on slides submitted by veterinarians from the United States, Canada, Mexico, Europe, Japan, and Australia. Comparative IFA confirmatory tests were conducted for 10 years between September 1, 1979 and August 31, 1989. Other comparative research IFA tests were performed at laboratory B.[b]

The IFA procedure was performed as described on blood smears mailed to the laboratory.[1,2,18,44,45] Good quality, thin feathered-edge, blood smears were fixed for 7 minutes in absolute methanol at 20 to 22 C (room temperature). Reaction wells (1.5-cm diameter) were drawn, using a china marker pencil, on the leading edge of the blood smears. Rabbit anti-FeLV serum, at dilution of 1:60, was placed in the reactive wells and slides were tested as described.[1,45] Each FeLV test was performed with 2 positive-control blood smears included.

ELISA procedure—All comparative FeLV ELISA were performed by veterinarians in their hospitals or by local veterinary diagnostic laboratories, using all of the commercially available ELISA kits (Tables 2 and 3). Blood smears from the 20,240 cats that were tested by ELISA were submitted by veterinarians to laboratory A for IFA test confirmation between September 1, 1979 and August 31, 1989 (Table 3).

Comparison of ELISA and IFA discordant results with tissue culture isolation of FeLV—Three discordant ELISA-positive, IFA-negative cat sera and 1 dis-

Table 3—Overall ten-year comparison of FeLV ELISA and IFA test by individual ELISA kit: September 1979 through August 1989*

ELISA test kit	ELISA result	No. tested	IFA result		Percentage agreement (%)
			Negative	Positive	
Kit A	Negative	627	545	82	86.9
	Positive	8,492	5,210	3,282	38.7
Kit B	Negative	248	222	26	89.5
	Positive	2,326	612	1,714	73.7
Kit C	Negative	54	50	4	92.6
	Positive	1,280	792	488	38.1
Kit D (saliva)	Negative	25	21	4	84.0
	Positive	328	226	102	31.1
Kit E	Negative	89	77	12	86.5
	Positive	1,747	816	931	53.3
Unknown ELISA	Negative	51	46	5	90.2
Local laboratories	Positive	1,361	634	727	53.4
In-hospital	Negative	169	134	35	79.3
	Positive	1,968	1,030	938	47.7
	Negative	1,332	1,158	174	86.9
Total	Positive	18,908	10,147	8,761	46.3
		20,240	11,305	8,935	49.0

Overall agreement: Results for 9,919 of 20,240 (49.0%) total tests agreed.

IFA tests performed by laboratory A.
This study was biased toward positive ELISA results confirmation.

*Table 4—Comparison of discordant ELISA and IFA results with isolation of FeLV in tissue culture**

Discordant FeLV results		Tissue culture isolation		
		No. Tested	Results	
ELISA	IFA		Positive	Negative
Positive	Negative	3	3	0
Negative	Positive	1	0	1

*100% agreement between IFA and tissue culture isolation results.

[a]National Veterinary Laboratory Inc, Franklin Lakes, NJ.

[b]Laboratory of Veterinary Oncology, Memorial Sloan Kettering Cancer Center, New York, NY.

Table 3—Retesting of 48 FeLV ELISA-IFA discordant cats

Initial FeLV test results		Retests of discordant cats			
		No change of results—Retest Results		Change of results Retest results	
ELISA + IFA	ELISA - IFA +	ELISA + IFA +	ELISA - IFA +	ELISA + to IFA to	ELISA - to IFA to +
47	1	13 (27.1%)	1 (2.1%)	34 (70.8%)	0 (0%)
48					

Table 4—Comparative sensitivities of FeLV tests

Dilution of blood* in saline solution	Comparative tests		
	Immunodiffusion	IFAT	ELISA‡
Undiluted	-	-	-
1:10	-	-	W
1:100	-	-	
1:1,000	-	W	

*Blood from 2 FeLV positive cats. †Performed by laboratory A. ‡Kit B. + = positive. - = negative. W = weak reaction.

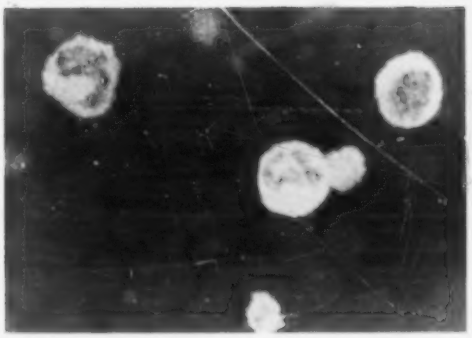

Figure 1—Positive immunofluorescent antibody (IFA) test result on a blood smear from a cat. The FeLV antigens are in neutrophils, lymphocytes, and platelets.

cordant (ELISA negative, IFA positive) cat serum were tested for FeLV by tissue culture isolation (TCI) (Table 4). Sera were obtained fresh and were inoculated within 3 hours onto feline embryo fibroblast (FEA) cell cultures.[3]

Retests of ELISA-IFA discordant cats—A group of 48 ELISA-IFA discordant cats were retested by ELISA and IFA within 1 week after obtaining original ELISA-IFA discordant test results (Table 3). The same ELISA kit[2] (kit A) that was used for the original tests was used to retest each cat, and blood smears were prepared at the same time for confirmatory IFA retesting.

Comparative sensitivities of FeLV tests—Relative sensitivities of various FeLV immunodetection tests were evaluated by obtaining sera and blood smears from 2 FeLV-infected cats. Each serum was tested by immunodiffusion (ID)[3,4,5] and ELISA[2] (kit B) undiluted and at various dilutions in phosphate-buffered saline solution (PBSS): 1:10, 1:100, and 1:1,000 (Table 4). Blood smears were prepared and tested by IFA after blood samples were diluted in PBSS.

[3]Leukassay-F, Firman-Moore, Inc., Mundelein, Ill.
[4]CITE, Idexx Corp, Portland, Me.

Figure 2—Negative IFA test result on a blood smear from a cat. No FeLV antigens are in the neutrophils and lymphocytes.

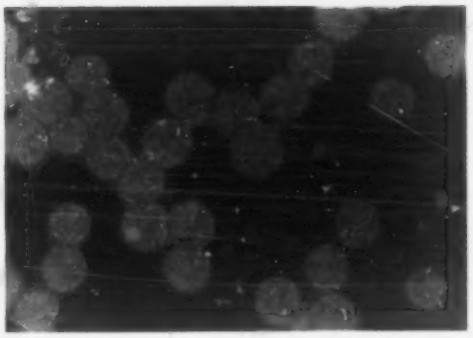

Figure 3—An FeLV IFA test on blood smears from a cat with severe nonregenerative anemia. Leukocytes are not on the smear, and thus, examination is not possible.

Results

IFA testing—The IFA test for FeLV detects FeLV antigens as apple-green punctate fluorescent granules in the cytoplasm of neutrophils, eosinophils, lymphocytes, and platelets in blood smears (Fig 1).[6,7] All leukocytes are FeLV antigen-positive in most IFA-positive cats. Occasionally however, only 10 to 90% of the leukocytes will be positive for FeLV antigens. In this instance, the IFA test is positive for FeLV infection and the veterinarian is advised to recommend isolation of the cat and to retest the cat in 1 month. The 1-month retest is suggested to determine whether the cat's leukocytes will become 100% antigen-positive or 100% antigen-negative. Cats with <100% IFA antigen-positive leukocytes are either in the early stages of infection or are in the act of rejecting FeLV infection and becoming immune. The ELISA are not able to identify this type of early infection or rejection of the virus.

No fluorescence is seen in noninfected cat leukocytes, which appear red because of the Evans blue counter stain (Fig 2). The IFA test is difficult to

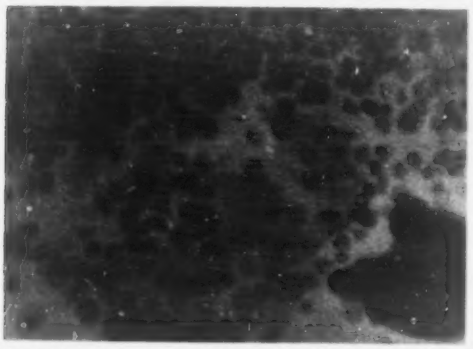

Figure 4—An IFA test on a blood smear that is too thick. This causes a no-evaluation result because of nonspecific reaction.

interpret in cats that are leukopenic because of low numbers or lack of leukocytes (Fig 3). Blood smears that are too thick are also difficult to interpret because of lack of visible leukocytes within the heavy cell layer and because of the nonspecific reaction attributable to the trapping of the IFA reagents (Fig 4).

Between June 1, 1972 and December 31, 1990, laboratory A performed 1,142,600 IFA tests (Table 1), of which 19.8% had positive results and, 78.0% had negative results; in 2.2%, evaluation was not possible because of lack of leukocytes in the smears or nonspecific staining reaction. A higher percentage of positive results was obtained from 1972 through 1978 than in more recent years, from 1979 through 1990.

IFA and ELISA comparative testing—For 10 years, between September 1, 1979 and August 31, 1989, laboratory A performed IFA confirmatory testing of samples from 20,240 cats that had been tested for FeLV by in-hospital ELISA (Tables 2 and 3). All 6 commercially available ELISA kits were compared with the IFA test.

As can be seen from the data in Table 2, the overall concordance of ELISA and IFA tests was only 49%. However, it should be noted that most (93%) of the confirmatory tests that were requested by veterinarians were for positive ELISA results, which introduced bias toward confirmation of positive ELISA results. There was 46.3% agreement between positive ELISA results and positive IFA results. The concordance for negative ELISA results was higher, with 86.9% agreement. However, this indicates that 13.1% of negative ELISA results were positive by IFA testing.

Concordance between ELISA results of each manufacturer's ELISA kit and the IFA test was calculated (Table 3). The ELISA kit with the highest concordance for FeLV-positive results was kit B[d] with 73.7% agreement, whereas the ELISA kit with the highest concordance for FeLV-negative results was kit C[e] with 92.6% agreement. The ELISA with the lowest concordance for FeLV-positive results were those used for testing saliva (kits C[e] and D[f]) with agreement of only 31.1 and 38.1%, respectively.

The ELISA with the lowest concordance for FeLV-negative results were also used for testing saliva (kits D[f] and E[g]) with agreement of 84.0 and 86.5%, respectively.

*Table 7—Calculation of the sensitivity and specificity of in-hospital ELISA**

ELISA results	Reference Standard results	
	Positive	Negative
Positive	a = 8,761	b = 10,147
Negative	c = 174	d = 1,158

Let a = IFA/ELISA-positive; b = IFA-negative, ELISA-positive; c = IFA-positive/ELISA-negative; d = IFA/ELISA-negative results. Total tested = a + c + b + d = 20,240. Overall agreement = 49.0%.

*Data biased toward confirmation of positive ELISA results; thus, these specificity data do not represent true unselected values.

Calculations:

$$\text{ELISA Sensitivity} = \frac{a}{a + c} \times 100 = \frac{8,761}{8,761 + 174} \times 100 = 98.1\%$$

$$\text{ELISA Specificity} = \frac{d}{b + d} \times 100 + \frac{1,158}{10,147 + 1,158} \times 100 = 10.2\%$$

$$\text{ELISA false negative rate} = \frac{c}{a + c} \times 100 = \frac{174}{8,761 + 174} \times 100 = 2.0\%$$

$$\text{ELISA false positive rate} = \frac{b}{b + d} \times 100 = \frac{10,147}{10,147 + 1,158} \times 100 = 89.8\%$$

Analysis of these data indicated that overall sensitivity (Table 7) of the ELISA was 98.1%, specificity was 10.2%, false-negative rate was 2.0%, and false-positive rate was 89.8%. These findings are distorted because more positive ELISA results were confirmed than were negative ELISA results. However these data clearly document that numerous false-positive in-hospital FeLV ELISA results are common.

Comparison of ELISA and IFA discordant results with TCI—Results of FeLV TCI agreed with IFA results in all 4 of the ELISA and IFA discordant cats tested (Table 4).

Retesting of ELISA/IFA discordant cats—Forty-eight ELISA/IFA discordant cats were retested by veterinarians within 1 week after results were initially obtained (Table 5). Of the 48 cats, 47 were initially ELISA-positive, IFA-negative, whereas 1 was initially ELISA-negative, IFA-positive. The ELISA and IFA retest results of 13 of the 47 initially ELISA-positive, IFA-negative cats and the 1 initially ELISA-negative, IFA-positive cat did not change. However, the ELISA results of retesting of 34 of the 47 (70.8%) initially ELISA-positive, IFA-negative discordant cats changed to ELISA-negative, whereas the IFA results remained unchanged and were still negative. These comparative retest results indicate that 70.8% of the initial ELISA results for cats in the ELISA/IFA discordant group were incorrect.

[e]Virachek, Synbiotics Inc, San Diego, Calif.

[f]ClinEase, Norden Laboratories, Lincoln, Neb.

[g]Diasystems, TechAmerica Veterinary Products, Kansas City, Mo.

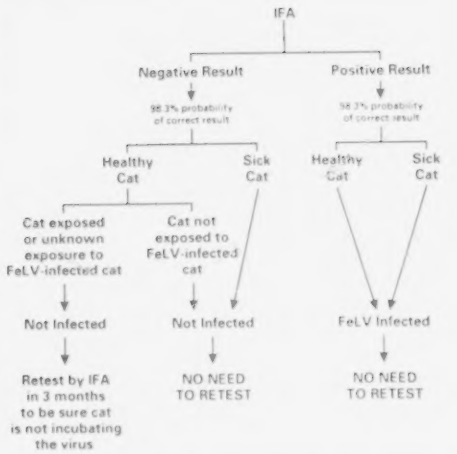

Figure 5—Algorithm for management of cats tested for FeLV infection by the immunofluorescent antibody (IFA) test.

ELISA
Negative Result
Positive Result
86.9% probability of correct result
46.3% probability of correct result
Healthy Cat
Sick Cat
Healthy Cat
Sick Cat
Cat exposed or unknown exposure to FeLV-infected cat
Cat not exposed to FeLV-infected cat
13.1% probability of incorrect test result
Not Infected
Not Infected
Not Infected ?
Infected ?
Retest by ELISA and IFA in 3 months to be sure cat is not incubating the virus
No need to retest
CONFIRM BY IFA IMMEDIATELY

Figure 6—Algorithm for management of cats tested for FeLV infection by ELISA.

Comparative sensitivities of FeLV tests—Sera and blood from 2 FeLV-positive cats were diluted and tested by ID, IFA, and ELISA[4] (Table 6). The ID test result was positive only when undiluted sera were tested, the ELISA result was weakly positive for sera diluted 1:10, and the IFA test result was weakly positive for blood diluted 1:1,000. In other experiments (not described here), it was possible to consistently detect, by use of the IFA test, 1 FeLV-infected leukocyte mixed with 100,000 noninfected leukocytes.

Discussion

This study was initiated by veterinarians who submitted samples for IFA testing for confirmation

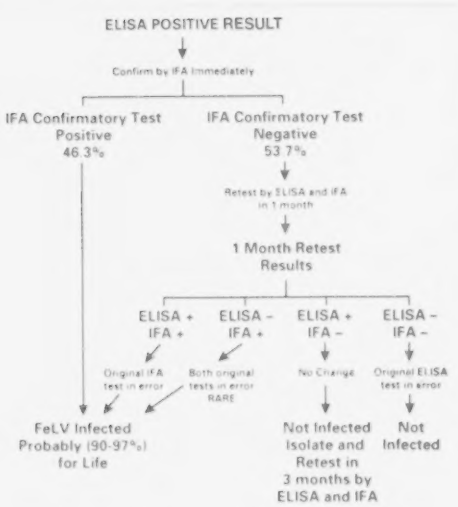
ELISA POSITIVE RESULT
Confirm by IFA Immediately
IFA Confirmatory Test Positive 46.3%
IFA Confirmatory Test Negative 53.7%
Retest by ELISA and IFA in 1 month
1 Month Retest Results
ELISA + IFA +
ELISA – IFA +
ELISA + IFA –
ELISA – IFA –
Original IFA test in error
Both original tests in error RARE
No Change
Original ELISA test in error
FeLV Infected Probably (90-97%) for Life
Not Infected Isolate and Retest in 3 months by ELISA and IFA
Not Infected

Figure 7—Algorithm for management of cats tested for FeLV infection by ELISA and confirmed by IFA testing.

of results of ELISA performed in their hospitals or at veterinary diagnostic laboratories. During the 10 years of this study, 20,240 confirmatory IFA tests were performed. Agreement between in-hospital performed FeLV ELISA and the IFA test was 86.9% for negative ELISA results and was 46.3% for positive ELISA results. It should be noted that 93% of all of samples submitted by veterinarians for confirmation had positive ELISA results, which introduced bias in the test selection. Practitioners apparently believed that their negative ELISA results were more reliable than their positive ELISA results. This assumption was correct, although 13.1% of all negative ELISA results were incorrect. These findings indicate that veterinarians should be more aware that even their negative ELISA results may be incorrect.

When the 4 discordant ELISA/IFA results were compared with TCI of the virus, all 4 TCI results agreed with the IFA test result. When samples with ELISA/IFA discordant results were immediately retested by ELISA and the IFA test, 70.8% of the original ELISA results changed and, thus, had been incorrect. In this retesting program, none of the IFA test results changed. The sensitivity of the IFA test was found to be higher than that of the ELISA when studied in dilution experiments. Results indicate that the IFA test is still the most accurate FeLV test for detection of persistent FeLV infection.

The biology of the virus and pathogenesis of FeLV infection have been elucidated by use of the IFA test.[1-19] In contrast, the biology of FeLV in ELISA-positive pet cats has not been extensively studied and the outcome of ELISA-positive, IFA-negative discordant cats is not known.[26-31] However, the biology of FeLV in ELISA (true)–positive cats should be identical to that of FeLV in IFA-positive cats.

The management of FeLV ELISA-positive cats is a substantial problem for veterinarians.[3,4,13,14,44,45,47] Conflicting recommendations have been made regarding the disposition and management of these cats. Some veterinarians recommend isolation of ELISA-positive cats in multicat households, others recommend that these cats be retested, by ELISA, in 1 month, and still others recommend euthanasia. Many veterinarians, however, recommend immediate IFA test confirmation of the positive ELISA result before making any decision concerning management of such cats.

We have developed 3 FeLV test algorithms to guide veterinarians for use with the FeLV IFA test and ELISA (Figs 5-7). The algorithm for the FeLV IFA test is based on studies that established the IFA test as the most accurate of FeLV tests (Fig 5).[1,45] There is 98.3% probability that an IFA test result is correct.[45] Thus, there is no need to retest cats that were tested by IFA, except IFA-negative cats that have been recently exposed to an FeLV infected cat or cats that have unknown exposure, such as stray cats. These cats should be retested 3 months after the first test to be sure they were not incubating the virus at that time.

The algorithm for FeLV ELISA also was developed (Fig 6). There is 86.9% probability that a negative ELISA result is correct. If the negative ELISA result is in a healthy cat exposed to an FeLV-infected cat, the former cat should be retested in 3 months by ELISA and the IFA test to be sure the cat was not incubating the virus. If the negative ELISA result is in a healthy cat that was not exposed to an FeLV-infected cat, there is no need for FeLV retesting. All sick cats with negative ELISA results, should have those results confirmed by the IFA test immediately if the veterinarian suspects FeLV-induced disease. There is only 46.3% probability that a positive ELISA result is correct. Thus, we recommend that positive ELISA results in all healthy and sick cats be immediately confirmed by IFA testing.

An algorithm for ELISA-positive IFA confirmatory testing was developed (Fig 7). In-hospital positive ELISA results are confirmed to be positive by IFA 46.3% of the time. Cats with such results are infected and 90 to 97% of them will remain infected for life. However, 53.7% of the positive ELISA results are not confirmed by IFA testing and thus, are negative. Cats with such results should be retested 1 month later by ELISA and IFA testing. If the IFA confirmatory test result is positive, the cat should be considered viremic and most likely will remain infected for life. However if the IFA confirmatory test result is negative, the veterinarian should inform the cat's owner of the discordant results and recommend retesting of the cat, using both tests, in 1 month. If the 1-month retest ELISA result changes from positive to negative but the IFA test result remains negative, the cat should be considered free of FeLV infection and the initial ELISA result should be considered erroneous. If the 1-month ELISA result is again positive and the IFA test result remains negative, the cat should be considered to be aviremic and not shedding the virus in the saliva. However if possible, these cats should be kept away from other cats and they should be retested once more, by ELISA and the IFA test, 3 months after the initial FeLV test.

Table 8—Summary of frequency of FeLV infections in stray or randomly selected cats classified by IFA test OR ELISA method

Region	Year	FeLV frequency (%)	No. of cats tested	FeLV test method	Reference
New York City and Boston	1973	0.3	638	IFA	2
Boston	1975	1.8	114	IFA	5
New York City	1981	1.0	1,290	IFA	18
San Diego	1981	0.6	158	IFA	18
Boston	1981	0	120	IFA	18
King County, WA	1981	4.9	1,451	IFA	54
Northwest US	1981	5.2	232	IFA	54
New York City	1990	2.1	4,191	IFA	*
Total IFA		2.3	8,194		
San Antonio	1984	13.6	44	ELISA	43
Hillsborough County, Florida	1984	9.4	555	ELISA	33
US and Canada	1989	13.3	1,609	ELISA	55
Japan	1989	12.4	700	ELISA	56
Southeast Texas	1990	18.0	156	ELISA	57
Total ELISA		12.7	3,064		

Adapted from reference 33.
*Data from National Veterinary Laboratory, Inc.

The FeLV ELISA-positive, IFA-negative discordant cats that retest ELISA positive and IFA positive at the 1-month retest should be considered viremic and the initial IFA test should be considered erroneous. In this case, the explanation that the IFA test result changed from negative to positive because the cat was incubating FeLV and the IFA test was less sensitive than the ELISA at detecting this early infection seems unlikely because of our ID, IFA, and ELISA comparative sensitivity studies. It is not desirable for any FeLV test to be so sensitive that it will detect early local tissue infection not accompanied by persistent viremia because it is known that some cats (42%) will reject initial local infection and become FeLV-free and immune.[10-12,15,18] Cats with latent FeLV infection do not have replicating FeLV in their tissues, and thus, these cats will not test positive by the IFA test or ELISA.[48,49,50] A practical and useful FeLV test is one that will only detect persistent viremia in cats that do not reject initial infection.

Feline leukemia virus tests should be used in conjunction with FeLV vaccination to prevent spread of this deadly virus among cats.[51,52,53] The FeLV test-and-removal program has been successful worldwide in eliminating the spread of FeLV in multicat households even before introduction of the FeLV vaccines.[3,4,13,14] However, we now recommend that all cats be inoculated with an FeLV vaccine in conjunction with an FeLV and FIV prevaccination screening program. Cats should be tested for FeLV and FIV at the initial office visit. Blood should be drawn for the FeLV and FIV tests,

and the first dose of the FeLV vaccine should be given. If the FeLV test result is obtained after the office visit is completed, it should be forwarded to the owner. It seems more economical to begin vaccination of cats with the first dose even before their FeLV status is known, because only 2.3% of random healthy cats, on the basis of IFA test surveys (Table 8), are expected to be infected with the virus.[15,16,18] The ELISA incidence data of random healthy cats are much higher than those compiled for the IFA test (Table 8), and we believe they represent an overestimate based on false-positive ELISA results that were not confirmed by IFA testing.[2,5,18,33,43,54,56]

Any cat that tests positive for FeLV after being given the first dose of the FeLV vaccine should be isolated from other cats and, although the vaccine is not harmful to infected cats, there appears to be no benefit to continuing the vaccination program in such cats. Healthy cats that test negative for FeLV and positive for FIV should be vaccinated for FeLV but should be kept indoors to prevent the spread of FIV through fighting and biting.[57] Because FeLV infection is life-long in 90 to 97% of IFA-positive cats and effective anti-viral treatment is not available, it is important to identify healthy FeLV carrier cats so they can be removed from contact with other cats.[58] The FeLV test-and-removal program is an excellent example of an efficient and effective anti-retroviral preventive veterinary medicine program.[3,4,13,14]

The percentage of positive IFA test results apparently decreased from 1972 to the present. A higher percentage of positive FeLV test results was observed in the years 1972 through 1978, than in more recent years. One explanation is that in the early 1970s, veterinarians were mainly testing sick cats or cats known to have been exposed to FeLV-infected cats. Another possibility is that, with the introduction of the first vaccine for FeLV in 1985, many veterinarians began prevaccination FeLV screening programs of more healthy FeLV-nonexposed cats. A review of 1,417 IFA test requisition forms from 1990 indicates that more healthy cats are currently being tested than were tested in the early 1970s. Currently, it is difficult to determine whether FeLV vaccines have reduced prevalence of FeLV-infected cats in the general population.

In this study, analysis of the comparison of practitioner-performed in-hospital ELISA with the IFA test performed by a specialized retrovirus testing laboratory indicated that more than half of positive ELISA results were incorrect. Results of this study are biased toward confirmation of a large number (n = 18,908), of positive ELISA results and only a relatively small number (n = 1,332) of negative ELISA results. However, our results indicate that many in-hospital positive ELISA results are incorrect and support the recommendation that all positive ELISA results be confirmed by IFA testing.

During the past several years, better correlation has been reported between ELISA and other IFA and virus-isolation tests done in university research laboratories.[18,20-44] Unlike our study, none of those studies compared large numbers of in-hospital performed ELISA. The probable explanation for the differing results between those comparative studies and our study is the fact that all comparative tests, ELISA and confirmatory tests, were performed by skilled laboratory-trained technicians in the other studies and not by veterinary hospital personnel as was associated with our study. Our study encompassed more than 1,000 veterinary hospitals where there probably were differing degrees of technical laboratory competence.

Many clinically oriented reviews exist of the biology of FeLV and the diseases induced by this virus.[3,17,18,47-59,60] Veterinarians in small animal practice, where cats now comprise more than half of all patients, must familiarize themselves with this virus and the methods used to detect infected cats. Veterinarians must also fully understand the characteristics (strengths and weaknesses) of the FeLV tests they use, so that the information gained from these tests can be used properly. An FeLV test is for detection of the virus and is not a diagnostic test for leukemia or any other feline disease.[44,45]

Careful confirmation of all positive retroviral test results should be done to practice the highest standards of veterinary medicine. In this regard, results of all retroviral screening ELISA for the human immunodeficiency virus types 1 and 2, and the human T-lymphotropic retrovirus types I and II are confirmed by more specific western blot assays before any person is informed of positive retroviral status. A similar standard should be adopted in veterinary medicine where routine retroviral testing was introduced and practiced 10 years before similar tests were available in human medicine.

As a result of this study, we recommend that all FeLV ELISA-positive cats have such results immediately confirmed by IFA testing.

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Comparison of four test kits for feline leukemia virus antigen

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Summary: *The sensitivity and specificity of 4 commercial FeLV ELISA kits, using blood, were compared with results of virus isolation from blood and immunofluorescent antibody (IFA) testing on blood. Significant differences were not found among the 4 ELISA kits. Marked decrease in sensitivity of the ELISA kits was detected when virus isolation was used as the standard of positivity rather than the IFA test. Virus isolation was a more sensitive indicator of early infection, with marked discrepancy among results obtained by virus isolation, ELISA, and the IFA test. Results became progressively more concordant as infection became fully established. Cats FeLV-positive by virus isolation alone were more likely to eliminate viremia. All cats FeLV-positive by IFA testing remained persistently viremic. Virus isolation, ELISA, and IFA testing appear to differ in their prognostic value. The use of blood rather than serum for the ELISA resulted in several discordant results. Six cats were FeLV-positive by ELISA when blood was tested but were FeLV-negative when serum was tested. Positive ELISA results were obtained for 4 of these cats when serum was tested, using extended incubation to increase sensitivity. It is possible that blood may actually be more sensitive than serum for use of the ELISA method.*

Detection of FeLV infection by use of an ELISA on serum is a sensitive, accurate, and simple procedure. Results of ELISA on serum were correlated (95%) to immunofluorescent antibody (IFA) detection of FeLV[1] on blood. Discrepancy usually is attributed to increased sensitivity of ELISA technology and to the ability of ELISA to detect infection prior to bone marrow involvement. False-negative IFA test results can be obtained when antigen concentration is low, when FeLV infection is sequestered outside the bone marrow, or when too few leukocytes are available to evaluate. False-positive ELISA results are attributable to improper washing technique or to anti-mouse immunoglobulin activity in the sample.[2]

In a recent study, 7 commercially available ELISA kits were compared using serum from known IFA-positive cats and FeLV-negative specific-pathogen-free (SPF) cats.[3] Since that report was published, several new or modified ELISA kits have been introduced on the market. To our knowledge, the sensitivity of FeLV ELISA kits, using a standard of virus isolation, has not yet been evaluated.

Many veterinarians use blood rather than serum when performing ELISA. Testing of blood may result in more false-positive reactions than does testing of serum.[4] False-positive results are attributed to the difficulty of adequate washing.[4] Serum samples were used in the previous study comparing ELISA kits.[3] To the authors' knowledge, direct comparison of specificity between use of blood and serum for ELISA kits has not been previously reported.

The objective of the study reported here was to compare the sensitivity and specificity of 4 commercially available FeLV ELISA kits, using blood, with IFA and virus isolation methods.

Materials and Methods

Commercially available ELISA kits—Four FeLV

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